RF Project 1492
~~Report No. 2~~

[redacted]

SEMI-ANNUAL

REPORT

by (NASA CR-51657)

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

Columbus, Ohio

To: NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
Washington 25, D.C.

12 (NASA Grant No. NsG-295-62; RF Proj. 1492)

On: BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF SMALL MAMMALS TO CLOSED GASEOUS ENVIRONMENT (Semi-Annual)

For the period: (Report 2) 28 Feb~~ruary~~ 1963 - 31 Aug~~ust~~ 1963

Submitted by: E. P. Hiatt
Department of Physiology

Date: 22 August 1963

[redacted]

GPO PRICE $ _____

OTS PRICE(S) $ _____

Hard copy (HC) 1.00

Microfiche (MF) .50

BIOLOGICAL EFFECTS OF PROLONGED EXPOSURE OF SMALL MAMMALS
TO CLOSED GASEOUS ENVIRONMENTS

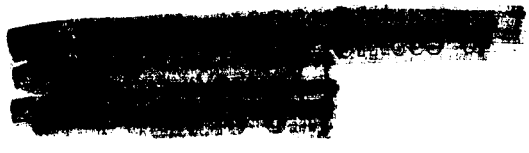
As indicated in our first status report of April, 1963, this program consists of several projects related to the general problem of the influence of the gaseous environment on animals. Some of these are major efforts which will result in papers to be published in scientific journals, others are smaller exploratory projects serving the double purpose of giving young investigators experience and providing leads for future major projects. Our progress in these several efforts is summarized briefly under separate headings below.

1. Development of chicken embryos and growth of young chickens in an atmosphere of approximately 79% helium and 21% oxygen

We now have completed two experiments in which fertilized chicken eggs have been incubated to hatching in an atmosphere almost devoid of nitrogen and with helium constituting the inert gas. Oxygen and total pressure were the same as in ordinary air at one atmosphere. In both experiments the hatchability of the eggs was reduced to about half of that of control eggs incubated in air in an identical closed chamber with the same controlled temperature, humidity, and CO_2 concentration (Table I). However the chicks which hatch in the helium-oxygen atmosphere grow at the same rate as their controls when maintained in the mixture of helium and oxygen for the period of observation, which extended to four week (Fig. 1). There is evidence that the chicks in the helium-oxygen atmosphere have a greater problem in maintaining their body temperature because of the relatively high thermal conductivity of helium. One sign of this is that they consume more food than the controls for the same rate of growth (Fig. 2).

Observations have also been made on heart rate, respiratory rate, body temperature, and oxygen consumption, but we shall defer reports on these observations since we are still improving our methods.

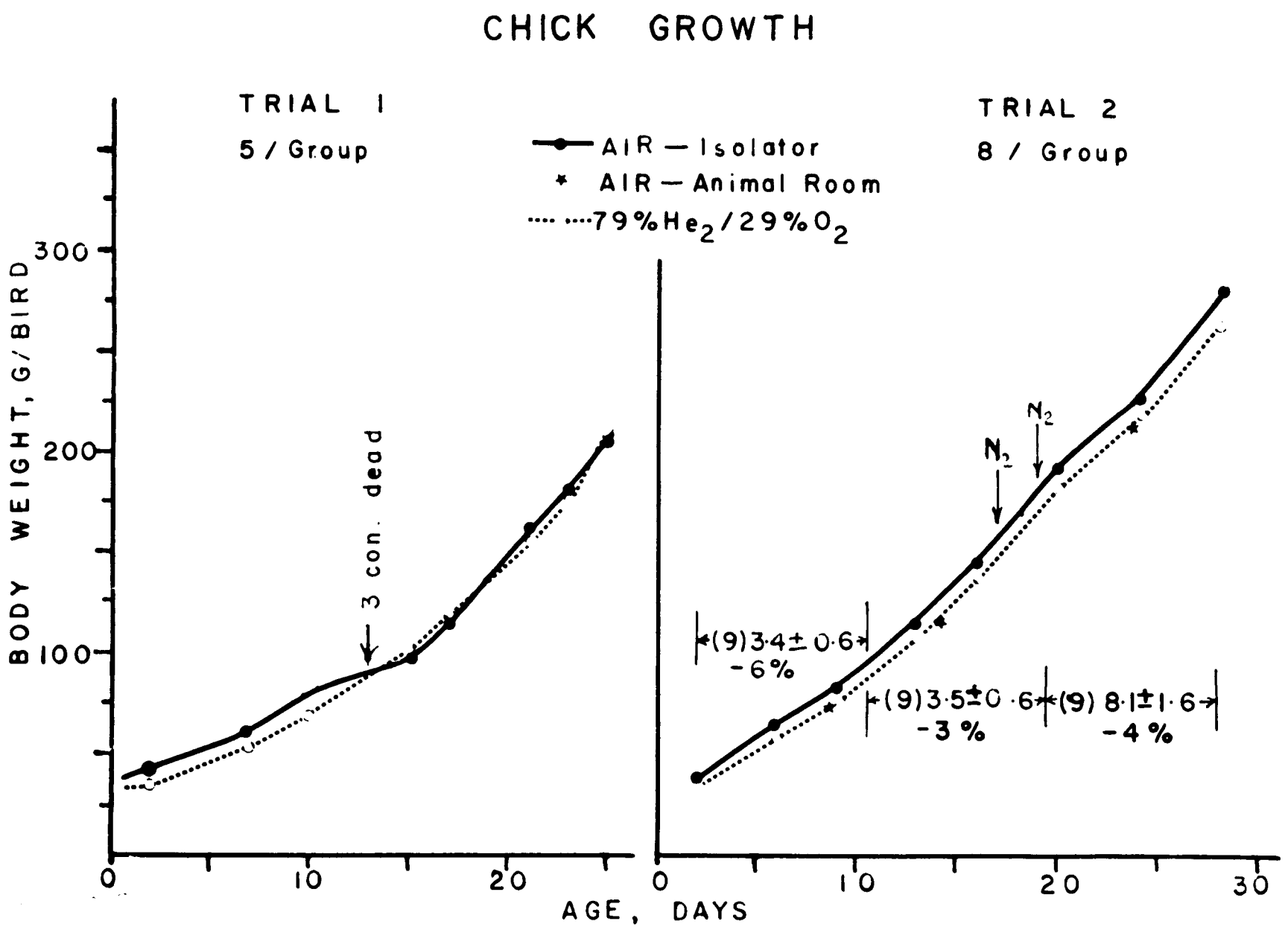
A report of this work was made at the Fall Meeting of the American Physiological Society at Coral Gables, Florida in late August.



HATCHABILITY

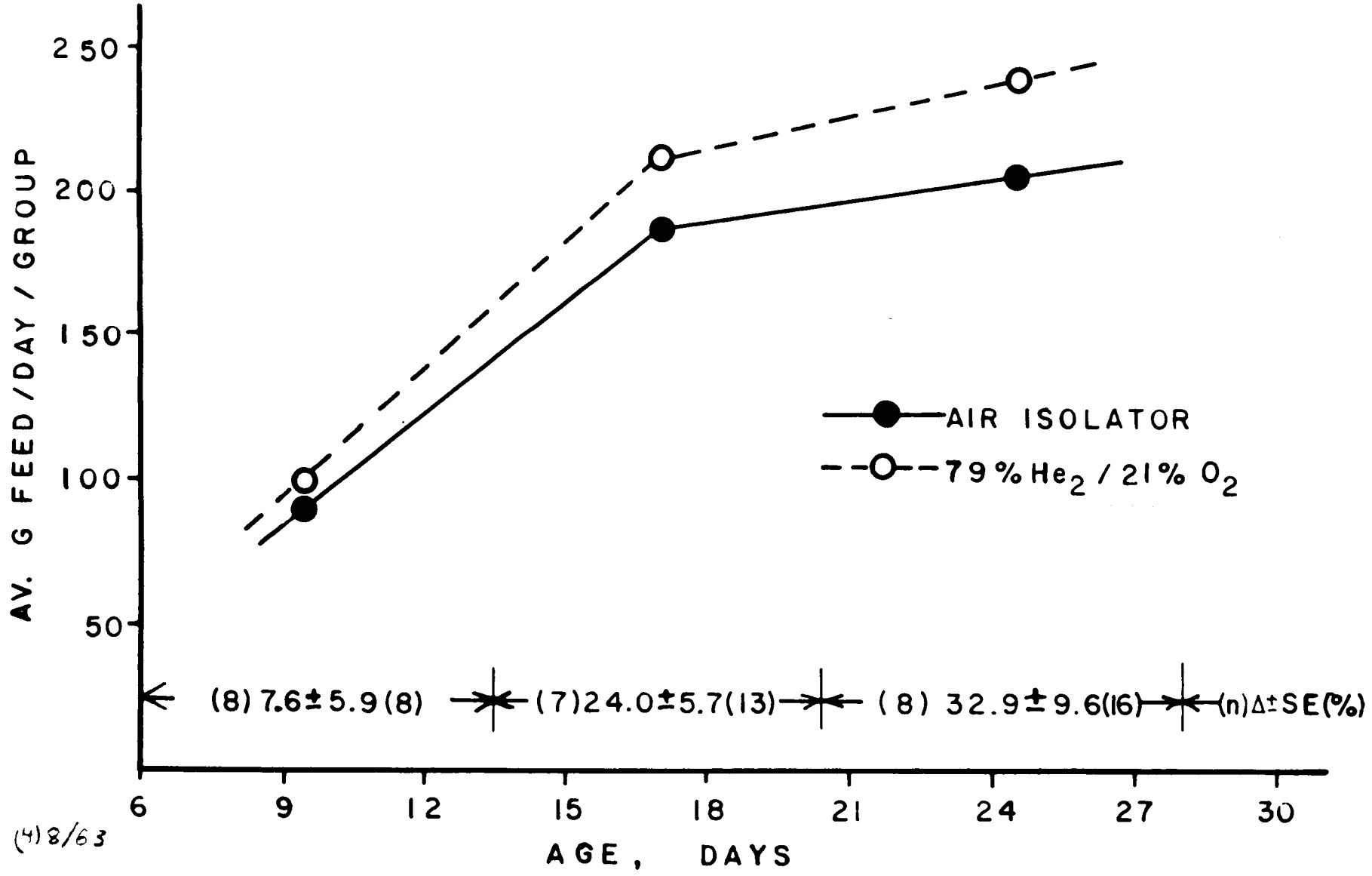
	TRIAL 1			TRIAL 2		
	AIR	79% He_2 21% O_2	SE or Chi Sq.	AIR	79% He_2 21% O_2	SE or Chi Sq.
No. EGGS SET	18	18		35	36	
No./% HATCHED	16/89	7/39	25.6**	20/57	8/22	17.7**
No./% INF. EARLY DEAD	—	—	—	6/17	10/28	3.2
No./% LaTE DEAD	—	—	—	8/23	16/44	10.3**
CHICK WEIGHT	(7)37.0	(7)33.8	±0.8**	(8)39.5	(8)36.1	±1.0*

(1) 8/63


CHICK GROWTH
TRIAL 1
5 / Group
AIR — Isolator
AIR — Animal Room
79%He2 / 29%O2
TRIAL 2
8 / Group
BODY WEIGHT, G/BIRD
300
200
100
3 con. dead
N2
N2
(9)3·4 ± 0·6
-6%
(9)3·5 ± 0·6
-3 %
(9) 8·1 ± 1·6
-4 %
0
10
20
0
10
20
30
AGE, DAYS

FEED INTAKE

(TRIAL 2 — 8 CHICKS/GROUP)



(4) 8/63

2. Development of chicken embryos in an atmosphere of almost pure oxygen at a reduced total pressure

We have been attempting to incubate fertilized chicken eggs in an altitude chamber filled with oxygen and kept at a sub-atmospheric pressure of about 196 mm Hg so that the partial pressure of oxygen in the eggs is near that of the controls incubating in air. Because of some unavoidable leaks in the chamber it was necessary to maintain a flow of oxygen through this chamber in order to keep the nitrogen below 2%. In early experiments we found that unless such eggs were kept at a very high humidity they lost weight rapidly because of increased rate of evaporation in the rarefied atmosphere. However, such embryos were always smaller than their controls and we have not yet been able to hatch any.

Our experiments on this project have been interrupted by the occurrence of a fire in the chamber. We are now installing a better electrical system and a CO_2-generating fire extinguishing system sensitive to heat.

3. Effect of nitrogen-free atmosphere on tissue metabolism and sensitivity to irradiation

Dr. Ronald Wright, D.V.M., is working on this project with the collaboration of Professor Milton Lessler of the Department of Physiology. They are studying oxygen consumption of homogenates of chick embryos with the Warburg technique. (Dr. Wright is working toward his Ph.D. and will begin a NASA traineeship in September.) So far they have found little difference in the oxygen consumption of embryos incubated in the absence of nitrogen from control embryos incubated in air. Furthermore, little difference is noted in the immediate effect of X-ray irradiation between the controls and the experimental eggs. However the study is continuing with improving technique and we hope to study the delayed effect of irradiation as well.

Note 1: With regard to the above three studies we have been interested to note some related investigations reported in the Russian literature: (1) M. I. Volskii has reported evidence that eggs incubated in air appear to fix gaseous nitrogen. He also reported that eggs incubated in atmospheres in which nitrogen was replaced by helium, argon, or xenon died after a few days. Doklady Akademii Nauk SSSR-Translation published by the American Institute of Biological Sciences, Vol. 128: 895, 1960, (2) V. V. Boriskin et al reported in the same journal, Vol. 148: 392, 1962, that they were able to hatch some chicks (about 28%) from eggs incubated in a helium-oxygen atmosphere and to maintain them for two weeks.

Dr. Shannon Allen, Ph.D., Professor of Physiology at the Chicago College of Osteopathy, visited this laboratory August 13 and spent the day consulting with us. He has been working for some years on the influence of lack of nitrogen on chicken embryos and other living materials. In general, he has reported that, without at least 10% nitrogen in the atmosphere, chicken embryos do not develop normally although his observations have been limited to the first four days of development. A summary of his work will appear soon in the Aerospace Medical Journal.

Note 2: Some explanation may be called for to account for our present use of chicken embryos instead of using mammals as we originally proposed and as we have used in the past. The main point is that chicken embryos make very good subjects for screening a number of gaseous environments for deleterious effects. They have been studied intensively so that excellent standards exits for evaluating developmental deficits. Also, they are easily cared for and small enough so that statistically valid observations can be made in limited space. We intend to go back to mammals to validate any leads we may obtain in our work with chickens.

4. The blood flow to bone marrow and other organs following varying periods of intermittent hypoxia in the rat

Miss Phyllis Arscott has finished a Ph.D. thesis with the above title. She was supported to a small extent (supplies and materials and two months stipend) by funds from our grant and is preparing a paper for publication which will credit this source.

In summary, she caused a marked, hematopoietic response in rats by exposing them for several hours a day to a 10% oxygen-90% nitrogen atmosphere. After total periods of hypoxia ranging from 35 to 105 hours she measured cardiac output and blood flow to bone marrow, kidney, lung, spleen, adrenal, and bone with a technique utilizing radioactive rubidium. Although there was an increase in cardiac output and in blood flow in most of the tissues examined at the time of the hematopoietic response, the kidney and bone marrow did not show augmentation of flow. This work has interesting applications to the theories of bone marrow stimulation and control. The blood flow to the bone marrow was about o.7 ml per gm per min., which is a relatively high rate of flow when compared with other tissues.

5. Oxygen toxicity studies

We are still in the exploratory stage of this study but already some interesting leads are turning up. We intend to study the relationship between the toxic effect of various periods of exposure to pure oxygen at one atmosphere and the recovery which takes place in air after such an exposure. We are particularly interested in the effects on the lung and have assembled an experimental chamber and carried out a few experiments in which mice were kept in one atmosphere until they died after a few days. Studies have been initiated to establish the histology of lungs exposed to high oxygen tensions pre- and post-mortem. We are being helped in this by Dr. Philip Pratt of The Ohio State University Department of Pathology and the Ohio Tuberculosis Hospital, who has worked extensively in the field of pulmonary pathology associated with hyperoxia.

One interesting sidelight appears in the fact that chickens kept in the same chamber with the mice were still in apparent good health weeks after all the mice were dead. Whether this has anything to do with the fact that birds do not have the surfactant material in their lung, which is so important in mammals, remains to be determined.

It is probable that Captain Richard Pilmer, USAF, who is taking a Ph.D. in physiology with us, will work on some aspect of this problem for his Ph.D. thesis.

We have also employed part-time another graduate student, Mr. David Beckman, to help us with our histological studies. Mr. Beckman has experience in studying tissues with the electron microscope.

6. Gas exchange and cardiovascular effects in short periods of intensive isometric exercise in man

Dr. Robert Bartels, Ph.D., of The Ohio State University Department of Physical Education, has worked with us this past summer measuring the oxygen consumption, carbon dioxide output, blood pressure, and heart rate of human subjects before and after a ten-second period of isometric exercise. In this period they pulled on a dynamometer in such a way as to involve most of the body musculature at approximately 60% of maximal effort. It is thought that perhaps such a system might be a useful way for confined personnel such as astronauts to maintain not only muscle strength but also bone structure and cardiovascular reflexes. It has been observed that oxygen consumption may increase as much as 30 times as a result of such short exercise periods, though it may be spread out over the following five minutes. Furthermore, there were marked changes in blood pressure and pulse rate indicating excitation of cardiovascular nervous control. The peak rates of O_2 consumption and carbon dioxide output will have value in the design of gas supply and absorber systems.

Some experiments have been carried out with the subjects breathing a mixture of 21% oxygen and 79% helium but no marked effect has been noted.

This line of experimentation, together with some related work on indices of physical fitness, has proved to be so interesting we plan to initiate a separate proposal to expand this program. The present work is in preparation for publication.

RESUME

It is apparent that our project consists of several sub-projects, some of which extend beyond a strict interpretation of our original title. In addition to studies on the effect of almost nitrogen-free environments as stated in out title, we have studied oxygen toxicity, hypoxia, and effects of isometric exercise in air and other atmospheres. [redacted] All of the projects except the one on exercise in man can be included under a title such as Biological Effects of Unusual Gaseous Environments amd perhaps we should change to this title.

The exercise project, including some work on indices of physical fitness, will form the basis of a separate proposal.

This report is given in condensed form without extensive data, bibliography, etc. because three of the six projects are being prepared for publication and copies of the manuscripts will be sent to NASA at the time of submission and reprints will be provided later.

Investigator _____ Date _____

Supervisor Edwin P Hall Date Sept. 11, 1963

For The Ohio State University Research Foundation

Executive Director Robert C Stephenson Date 9/11/63